CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
20-920

Clinical Pharmacology and Biopharmaceutics Review

CLINICAL PHARMACOLOGY and BIOPHARMACEUTICS REVIEW
Division of Pharmaceutical Evaluation I

NDA 20-920 **SUBMISSION DATE:** January 9, 2001

Natrecor® (nesiritide) for Injection
Scios Inc.
Sunnyvale, CA **REVIEWER:** Angelica Dorantes, Ph.D.

TYPE OF SUBMISSION: Original Major Amendment: Sponsor's Response to Non-Approval Letter

SYNOPSIS:

Natrecor® is a human B-type natriuretic peptide (hBNP) developed by Scios for the short-term treatment of congestive heart failure. Original NDA 20-920 for Natrecor was submitted on April 24, 1998. The Agency found that the provided information was inadequate and a non-approval letter dated April 27, 1999 was issued for the NDA.

In this submission dated January 9, 2001, Scios is providing a major amendment in response to the Agency's April 27, 1999 non-approval letter for NDA 20-920 for Natrecor. Specifically, the Agency conveyed the following concerns:

- The need for additional safety data to better understand the onset and offset characteristics of symptomatic hypotension when Natrecor® is added to standard-care therapies in a typical hospital setting.
- Broader range of CHF patients, including those with active ischemia, preserved systolic function, and those receiving other IV vasoactive agents.
- The need for an active-controlled study: comparing Natrecor® to an IV vasodilator such as nitroglycerin to provide a clearer characterization of Natrecor's efficacy and safety profile, especially as it relates to effects on blood pressure and hypotension.
- Questions pertaining to symptom evaluations including the appropriateness of measuring symptoms in patients without dyspnea at rest and the potential bias created by physician evaluations and the knowledge of hemodynamics
- Data to support the sustained hemodynamic effects of Natrecor®.
- A trial to support the recommendations for the dosing regimen and dose adjustment.
- Unrestricted use of diuretics and other cardiac therapies.

Based on the above concerns, Scios designed the VMAC trial (Vasodilation in the Management of Acute Congestive Heart Failure). The results from the VMAC trial confirmed the efficacy demonstrated in the original NDA. The VMAC data also addressed the overall safety of Natrecor, including the occurrence of symptomatic hypotension. Thus, the VMAC trial addressed in full the issues outlined in the Agency's non-approval letter.

With respect to the "Human Pharmacokinetics and Bioavailability" section of the NDA, it should be noted that this major amendment did not include any information for section 6. All clinical pharmacology and biopharmaceutic information was submitted under the original NDA for Natrecor. Original NDA was reviewed in 1999 by Dr. Nakissa Sadrieh and deemed acceptable with the only

exception of the labeling that is being reviewed herein.

RECOMMENDATION:

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation I (OCPB/DPEI) has reviewed the labeling information included in this major amendment to NDA 20-920 for Natrecor dated January 9, 2001. OCPB is of the opinion that the proposed text included in the "Pharmacokinetics and Metabolism" subsection of the labeling should be revised as recommended below.

Sponsor's Proposed Text:

Pharmacokinetics and Metabolism

DRAFT LABELING

OCPB Recommended Text:

Pharmacokinetics

Elimination

Pharmacokinetics/Pharmacodynamics

Special Populations

Effects of Concomitant Medications

Please convey the above Recommendation as appropriate to the sponsor.

/S/ 6/8/01

Angelica Dorantes, Ph.D.
Division of Pharmaceutical Evaluation I
Office of Clinical Pharmacology and Biopharmaceutics

RD/FT Initialed by Patrick Marroum, Ph.D. _____

cc: NDA 20-920, HFD-110, HFD-860 (Dorantes, Mehta), and CDR (Biopharm).

Attachment I

Includes

NDA 20-920

Proposed Labeling for Natrecor[R] for Injection

16 pages redacted from this section of the approval package consisted of draft labeling

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
20-920

Biopharmaceutics Review

APR 15 1999

Biopharmaceutics/Pharmacokinetics Review

NDA: 20-920

Sponsor: Scios Inc.
Drug: Nesitiride (Natrecor).

Drug class: Natriuretic peptide.

Type of Submission: Sponsor's response.

Date of Submission: April 6th, 1999.

Reviewer: Nakissa Sadrieh, Ph.D.

Background:

This submission is the sponsor's response to comments made in the final Clinical Pharmacology/Biopharmaceutics review dated March 26th 1999. Enclosed are the responses of the firm regarding two statements made in the review.

The following is the OCPB response to the sponsor's comments:

1-The Ce50 referred to in the review is the concentration in the effect compartment that would result in a 50 % decrease in the maximal pharmacodynamic effect. Thus according to the model that was built using data obtained from the clinical trials, the EMAX for the hypotensive effects of Natrecor was estimated to be 21.2 mm of Hg. If one believes this model, one would expect to have a 10.6 mm of Hg reduction in systemic blood pressure (SBP) at a concentration of 3 ng/ml in the effect compartment. These points were raised in the review which was based on data submitted by Scios and including Dr. Sambol's Pk/Pd

analysis. It is acknowledged that the CE50 cited in the review should not have been referring to the plasma concentration of Natrecor.

2-Whether one believes the Pk/Pd model or not, in the clinical trial data base, some of the patients took up to 20 hours to recover from the observed hypotension. One might argue about the magnitude of the lag between the Pk and Pd with regards to hypotension. However, the fact remains that in emergency situations one would still have to wait several hours before a patient's SBP returns to baseline. This lag in effect does not provide the clinician with flexibility in dosing regimens for Natrecor. Similarly, dosing adjustments are not expected to be easily achieved.

Conclusion:

It is the opinion of the Office of Clinical Pharmacology and Biopharmaceutics, that the concerns raised by the sponsor do not impact on the reviewer's conclusions which were based on the Pk/Pd analysis undertaken by Dr. Sambol for Scios.

4/15/99.

Nakissa Sadrieh Ph.D.

RD/FT by Patrick J Marroum 4/15/99

CC: NDA 20-920, HFD 110, HFD 860 (Sadrieh, Mehta), CDER document room: Attn Barbara Murphy

MAR 25 1999

Biopharmaceutics/Pharmacokinetics Review

NDA: 20-920

Sponsor: Scios, Inc.

Drug: Natrecor (Nesiritide for injection)

Formulation and Strengths: 2.5 and 5.0 mg strengths to be reconstituted with 5% Dextrose or 0.9% NaCl.

Drug class: Recombinant human B-type natriuretic peptide (hBNP)

Type of Submission: Original application

Date of Submission: **April 24th, 1998**
December 4th, 1998
February 10th, 1999
February 22nd, 1999
March 12th, 1999
October 16th, 1998 (IND)

Reviewer: Nakissa Sadrieh, Ph.D.

Synopsis:

Natrecor is human B-type natriuretic peptide (hBNP) which has been developed by Scios for the short-term treatment of congestive heart failure.

An ELISA was used to quantitate the levels of endogenous and exogenous natriuretic peptide and the assay showed that a large variability was present amongst subjects.

Four IV bolus studies were conducted. Three studies were conducted in patients with congestive heart failure and one was in patients with postoperative pulmonary hypertension after coronary artery bypass

surgery. One of the IV bolus studies was carried out in CHF patients that were also taking enalapril. For the IV bolus studies, doses up to 25 μg/kg were given as a single dose and 10 μg/kg were given every 4 hours for 24 hours. Additionally, four IV infusion studies were conducted. For these IV infusion studies, doses up to 0.1 μg/kg/min for 1.5 hours were given and 0.03 μg/kg/min for 24 hours (after a 0.6 μg/kg IV bolus).

Briefly, the elimination of a bolus dose of Natrecor follows a 2-compartment model with the alpha phase accounting for 30% of the AUC. The estimate for the t ½α=1.4 minutes and t ½ß=20.2 minutes. The CL=9.8 ml/min/kg (7.8 ml/min/kg, 11.8 ml/min/kg). The Vss and CL values were not dose-related, indicating that the PK of Natrecor was linear. The PK estimates for Natrecor were not different when administered to CHF patients taking enalapril, as compared to patients that were not taking enalapril.

A population PK analysis was carried out to determine the effects of demographics and clinical variables on PK parameters. No correlation was found between the CL of Natrecor and the following patient variables: age, gender, race/ethnicity, baseline pulmonary capillary wedge pressure and cardiac index, baseline (endogenous) hBNP concentration, NYHA classification of CHF, serum creatinine, and estimated creatinine clearance. However, in study 704.325, there was a suggestion of a slight trend towards a positive relationship between CL and creatinine CL, and an inverse relationship between CL serum creatinine. A population PK study conducted with data from study 311 showed that Natrecor CL increased with increasing body weight. Therefore, body weight was a significant covariate for hBNP CL.

Data from studies 704.307 were analyzed using NONMEM. A saturation model (sigmoid Emax model) was determined to best describe the relationship between (predicted) steady state exogenous plasma hBNP concentration and each hemodynamic response tested. No apparent delay in response was noted after plasma hBNP concentrations had reached steady state. The analysis of hemodynamic responses at early (1 and 1.5 hours) and delayed (after 2.5 and 3.0 hours) times following escalation to the 0.03 μg/min/kg in 6 subjects suggested that in addition to the plasma levels of hBNP, pharmacodynamic responses had reached steady state. A direct relationship between steady state plasma concentrations of Natrecor and pulmonary capillary wedge pressure (PCWP), cardiac index (CI) and systemic vascular resistance (SVR) was found. The mean C50 for both PCWP and SVR was estimated to be 2400

infusion of 0.02 µg/min/kg. Based on the mean estimates of Emax for PCWP of −16 mm Hg in patients with Class II or III CHF and for SVR of −450 dynes.sec.cm^{-5}, this infusion rate is predicted to decrease PCWP by about 8 mm Hg and SVR (in the typical patient weighing 80.5 kg) by about 225 dynes.sec.cm^{-5}. Individuals weighing considerably more or less than 80.5 kg are predicted to require a dosage adjustment to achieve the same SVR. A slightly higher C50 of 3100 pg/ml (corresponding to an infusion rate of 0.03 µg/min/kg) was estimated for CI. Administration of this dose to a patient of typical weight would be expected to increase CI by 0.34 L/min/m^2.

Recommendation:

The NDA for Natrecor (Nesiritide) Injection fulfills the requirements of the Office of Clinical Pharmacology and Biopharmaceutics.

The comments listed in this document should be forwarded to the sponsor.

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Appendices

Appendix 1: Study 704.305. A Phase I/II double-blind, randomized, placebo-controlled ascending dose study of the hemodynamic and renal effects of a single intravenous bolus of Natrecor hBNP in subjects with congestive heart failure.

Appendix 2: Study 704.309. A Phase II randomized, double-blind, placebo-controlled dose response study of a 24-hour course of Natrecor hBNP administered as an intermittent intravenous bolus in subjects with congestive heart failure.

Appendix 3: Study 704.310. A Phase II, randomized, double-blind, placebo-controlled, ascending dose-response study of a 24-hour course of Natrecor hBNP administered as an intermittent intravenous bolus in subjects with congestive heart failure or angiotensin converting enzyme inhibitor therapy.

Appendix 4: Study 704.306. A Phase I/II, randomized, double-blind, placebo-controlled, ascending dose-response study of the hemodynamic, renal, and neurohormonal effects of a continuous infusion of Natrecor hBNP in subjects with chronic congestive heart failure.

Appendix 5: Study 704.307. A Phase II, randomized, double-blind, placebo-controlled, crossover study of the hemodynamic effects of an intravenous incremental-dose infusion of Natrecor hBNP in subjects with congestive heart failure.

Appendix 6: Study 704.311. A randomized, double-blind, placebo-controlled, multicenter, dose-ranging study to evaluate the safety and efficacy of a 24-hour intravenous infusion of Natrecor hBNP insubjects with congestive heart failure.

Appendix 7: Study 704.325. A randomized, double-blind, placebo-controlled study of two doses of Natrecor hBNP administered as a continuous infusion in subjects with decompensated CHF.

Appendix 8: Study 704.312. A dose-ranging study of Natrecor hBNP in the treatment of postoperative hypertension after coronary artery bypass surgery.

Appendix 9: Assay methodology

Appendix 10: Pharmacodynamic analysis of Natrecor hBNP: estimated with study 704.309 and predicted for studies 704.305 and 704.307.

Appendix 11: Pharmacokinetic-pharmacodynamic model of Natrecor hBNP: estimated with studies 704.305, 704.307 and 704.309 and predicted for study 704.311.

Background:

Natrecor is Human B-type Natriuretic peptide (hBNP). BNP is a naturally occurring cardiac hormone produced in the cardiac ventricle. It has vasodilatory, diuretic, natriuretic and neurohormonal effects. The drug has been developed by Scios Inc. as an intravenous agent for the short-term treatment of congestive heart failure.

The preparation contains purified peptide produced by recombinant DNA technology. However, initially, the drug was produced using a synthetic peptide methodology. Natrecor is a 32 amino acid peptide. The amino acid sequence is identical to the endogenous hormone.

Natrecor is recommended to be administered as a continuous intravenous infusion at a dose of 0.015 μg/kg/min (the dose can be pushed up to 0.03 μg/kg/min for better hemodynamic results, in patients that tolerate the 0.015 μg/kg/min dose). Continuous infusion is reported to provide a more consistent effect on the improvement of cardiac hemodynamics, as compared to repetitive bolus regimens (every 4-6 hours). However, bolus doses may be used for the treatment of acute pulmonary hypertension or postoperative hypertension. The duration of the IV infusion is reported to vary with the clinical status at presentation, underlying medical condition and responsiveness to therapy. In Phase III, Natrecor has been given for up to 9 days. However, 83% of the patients have received Natrecor for 72 hours or less.

Names

The USAN name for human B-type (brain) natriuretic peptide (1–32) (hBNP) is nesiritide. The proprietary name for the vialed, formulated, lyophilized form of hBNP is Natrecor® (nesiritide).

The Scios Inc. product code number for the bulk hBNP drug substance that has been manufactured to date is Z1016.

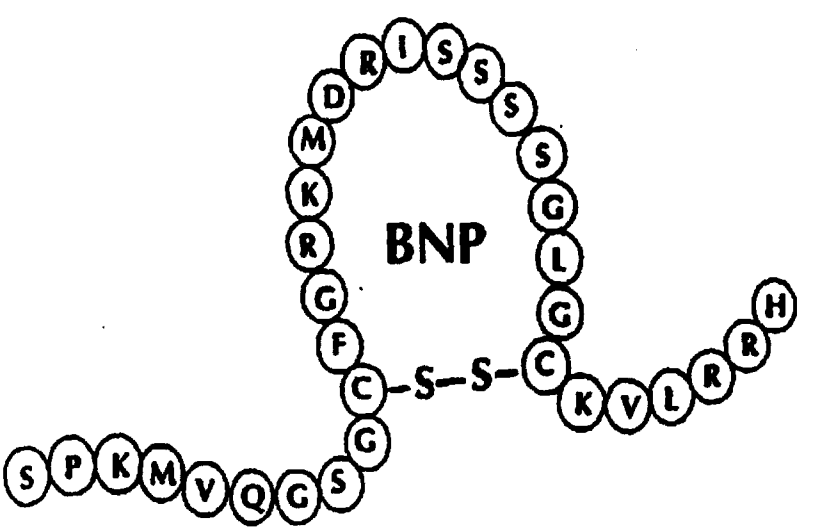
Physical and Chemical Characteristics

Human BNP is a 32–amino-acid peptide with the following amino acid sequence:

SerProLysMetValGlnGlySerGlyCysPheGlyArgLysMetAspArgIleSerSerSerGlyLeuGlyCysLysValLeuArgArgHis
1 5 10 15 20 25 30 32

A disulfide bridge connects the cysteines at positions 10 and 26, forming a ring of 17 amino acids with amino and carboxyl terminal extensions of 9 and 6 amino acids, respectively. A portrayal of the hBNP peptide nucleotide sequence is provided in Figure 3–1. The empirical formula is $C_{143}H_{244}N_{50}O_{42}S_4$ with a molecular weight of 3464 g/mol (average mass of base form).

Structure of Natrecor®



The contract manufacturer for the production of the drug substance is and for the drug product the manufacturer is

Natrecor can be reconstituted by the addition of 10 ml of 5% Dextrose or 0.9% NaCl. The withdrawal of 9.6 ml will yield 5mg of drug. The drug is also available in a 2.5mg dosage strength. This dosage strength contains half the amount of drug as the 5.0mg dosage form. The purpose of the 2.5mg vial is to minimize waste in the pharmacy. **The average amount of drug needed to prepare a 24-hour infusion at the recommended dose of 0.015 µg/kg/min is approximately 2.0 mg.** Therefore the advantage of the 2.5mg vial is clear. Therapy may continue for several days. A new vial will need to be reconstituted for each day of therapy.

Scios Inc. has obtained a waiver for the payment of PDUFA fees for Natrecor.

Summary of Results:

I. Single dose IV bolus study:

Study (704.305) was conducted in 30 patients with congestive heart failure (23 of which received Natrecor). The following doses of Natrecor were administered: 0.3, 1, 3, 10, 15 and 20 µg/kg. In this study, data from half the subjects were described by a 2 compartment model, whereas the data from the other half of the subjects were described by a one compartment model. In those subjects to whom a two-compartment model was applied the following PK parameters were reported:

$t_{1/2\alpha}$ =1.4 minutes (SE=0.3)
The alpha phase concentration accounted for 30% of the total AUC
The estimate of $t_{1/2\beta}$ =20.2 minutes (SE=1.8 minutes)
The estimate of Vc was 0.072L/kg (SE=0.010 L/kg)
The estimate of Vss was 0.130 L/kg (SE=0.018 L/kg)
Clearance was estimated at 5.4 ml/min/kg (SE=0.4 ml/min/kg)
There was no dose-dependence in Vss or CL

II. Intermittent IV bolus study:

Study 704.309 was an intermittent IV bolus study in which 44 subjects with congestive heart failure were administered 5 μg/kg of Natrecor every 4 hours or 10 μg/kg Natrecor every 4 or 6 hours (16 subjects received placebo). The mean parameter estimates, which were calculated by a model-independent (non-parametric) method, were the following:

CL:	9.8 ml/min/kg (7.8, 11.8 ml/min/kg)
Varea:	0.24 L/kg (0.20, 0.29 L/kg)
Vss:	0.21 L/kg (0.18, 0.24 L/kg)
MRT:	0.38 hours (0.34, 0.41 hours)
Parameter k:	Overall estimate: 2.55 h^{-1} (2.38, 2.71h^{-1})
Half-life:	Overall estimate: 0.29 hours (0.28, 0.31 hours)
Cmax:	Overall estimate: 37.4 ng/ml (32.7, 42 ng/ml)

No significant difference noted in the estimates of the first and last dose parameters. The sponsor reports that since the t ½ is 20 minutes and the dosing interval in the study was 4 or 6 hours (12-18 times the t ½), the plasma concentration at the end of the dosing interval is expected to be negligible. Therefore, since the difference between the first and last dose Cmax values is insignificant, this suggests that the central volume of distribution does not differ between the first and last bolus doses. Consequently, the lack of difference in the values for the first and last dose values for k and t ½ also suggests that clearance does not change over time with intermittent bolus dosing over 24 hours.

III. Drug interaction study with enalapril:

Study 704.310 was carried out because of reports of possible effects of ACE on the plasma elimination kinetics of porcine BNP on rats, where it had been reported that captopril increased the half-life and also the steady state concentration of porcine BNP from a continuous IV infusion. Therefore, Natrecor was administered as an intermittent intravenous bolus at doses of 3, 5 and 10 μg/kg every 4 hours for 24 hours to 41 subjects with congestive heart failure, 31 of which were also taking ACE inhibitors (specifically enalapril). Seventeen subjects were assigned to placebo, 13 of which were also receiving an ACE inhibitor. The following parameters were determined:

CL:	9.89 ml/min/kg (7.99, 11.80 ml/min/kg)
Varea:	0.26 L/kg (0.20, 0.33 L/kg)
Vss:	0.24 L/kg (0.18, 0.30 L/kg)
MRT:	0.40 hours (0.27, 0.33 hours)
Parameter k:	2.39 h^{-1} (2.19, 0.26 h^{-1})
Half-life:	0.30 hours (0.27, 0.33 hours)
Cmax:	33.4 ng/ml (18.6, 48.2 ng/ml)

Concomitant administration of enalapril was not found to affect the PK profile of Natrecor.

IV. Intravenous bolus study in patients with postoperative hypertension after coronary artery bypass surgery:

Study 704.312 was a bolus dose study of Natrecor at 5, 10, 15, 20 and 25 µg/kg/min administered to 24 patients with pulmonary hypertension after coronary artery bypass surgery. In all 24 patients, the data were fit to a 2-compartment model. The PK estimates obtained were as follows:

CL (ml/min/kg)	9.85 ± 0.76
α (min^{-1})	0.537 ± 0.124
t ½ α (min)	2.35 ± 0.30
(A/α)/(A/α+B/ß)	34.0 ± 4.0 %
ß (h^{-1})	2.71 ± 0.23
t ½ ß (h)	0.307 ± 0.031
(B/ß)/(A/α+B/ß)	66.0 ± 4.0 %
k21 (h^{-1})	9.92 ± 2.07
Vc (L/kg)	0.75 ± 0.008
Vss (L/kg)	0.170 ± 0.018

These data were consistent with those obtained in subjects with CHF. Additionally, no correlation was found between CL, Vc or Vss and dose.

V. IV infusion studies

Four IV infusion studies were carried out. Two of those will be reported in the present section and the other two will be reported in the next sections (under PK/PD studies for 704.307 and population PK studies for 704.325).

Study 704.306 was aimed at determining the PK profile of Natrecor following IV infusion for 4 hours to 16 subjects with CHF. The doses

used in the study were 0.025 and 0.05 µg/kg. The CL values determined were 9.7 ± 1.5 ml/min/kg (mean ± SE) for the 0.025 µg/kg dose and 17.3 ± 3.1 ml/min/kg (mean ± SE). The difference between these clearances approached significance (p=0.0509), but the sponsor argued that the presence of an outlier in the 0.050 µg/kg group did not permit drawing the conclusion that in fact the two treatment groups tended to have different clearances. However, the reviewer calculated the median for the clearance values in both dose groups and these were not very different from the means (9.05 and 15.8, for the low and high dose group medians, respectively). This indicated that in fact, the outlier was not solely responsible for the difference in clearances observed between the 2 dose groups, and that there may well be a real difference between the dose groups. It should also be noted that the sample size was rather small (n=6 in each group). The overall CL for the 2 treatment groups was 13.5 ± 2.0 ml/min/kg. The Vd was not different between the 2 dose groups, however the t ½ values for the low dose group tended to be higher than those for the higher dose group. The average t ½ was 19 ± 4 minutes (mean ± SE) and the average Vd was 0.26 ± 0.03 L/kg. A large amount of variability among subjects was noted in endogenous plasma levels of hBNP, as well as in the clearance of exogenous hBNP.

Study 704.325 was a continuous infusion study of Natrecor at 0.015 and 0.03 µg/kg/min, following a loading dose of either 0.3 or 0.6 µg/kg/min. The clearance of Natrecor at 6 hours after the start of infusion was estimated at 8.51 ml/min/kg in 127 subjects with congestive heart failure. The values of the clearances at 24 hours after the start of infusion were much larger and approached significance. In fact, at 24 hours after the start of infusion, the clearance values were 19.31 ± 32.66 ml/kg/min. In one subject, the clearance value increased from 23.26 to 180.72 ml/kg/min, from the 6 hour to the 24-hour time point, at which point the subject was considered to be a treatment failure and subsequently switched to another therapeutic agent. However, the sponsor states that the clearance values for the 24-hour time point are not to be relied upon for several reasons. The first reason was that many data points were excluded from the analysis (40 subjects). Additionally, there was a possibility of changes in the levels of endogenous hBNP with the improvement in the health of the patients over the course of the 24 hours treatment with Natrecor, which for the sake of the calculations of CL was assumed to be a constant.

VI. PK/PD studies:

Human BNP plasma concentration was modeled as the sum of the concentration resulting from the infused hBNP and the estimated endogenous hBNP concentration (a constant). Only steady state concentrations were analyzed, because of the large number of aberrant, non-steady-state data. A one-compartment model was appropriate for the estimation of clearance. Data from studies 704.307 and 704.311 were analyzed using the NONMEM program. A saturation model (sigmoid Emax model) was determined to best describe the relationship between (predicted) steady state exogenous plasma hBNP concentration and each hemodynamic response tested. The sigmoidicity factor, γ, that best describes the steepness of the concentration versus response relationship was determined to be 1 and subsequently fixed in all the analyses. No apparent delay in response was noted after plasma hBNP concentrations had reached steady state. Therefore a model requiring an effect compartment was not necessary. The analysis of hemodynamic responses at early (1 and 1.5 hours) and delayed (after 2.5 and 3.0 hours) times following escalation to the 0.03 µg/min/kg in 6 subjects suggested that in addition to the plasma levels of hBNP, pharmacodynamic responses had reached steady state. A clear relationship between steady state plasma concentrations of Natrecor and pulmonary capillary wedge pressure (PCWP), cardiac index (CI) and systemic vascular resistance (SVR) was found. The mean C50 for both PCWP and SVR was estimated to be 2400 pg/ml. Assuming mean hBNP clearance, a steady state concentration approximately equal to the C50 may be expected after continuous infusion of 0.02 µg/min/kg. Based on the mean estimates of Emax for PCWP of –16 mm Hg in patients with Class II or III CHF and for SVR of –450 dynes.sec.cm^{-5}, this infusion rate is predicted to decrease PCWP by about 8 mm Hg and SVR (in the typical patient weighing 80.5 kg) by about 225 dynes.sec.cm^{-5}. Individuals weighing considerably more or less than 80.5 kg are predicted to require a dosage adjustment to achieve the same SVR. A slightly higher C50 of 3100 pg/ml (corresponding to an infusion rate of 0.03 µg/min/kg) was estimated for CI. Administration of this dose to a patient of typical weight would be expected to increase CI by 0.34 L/min/m^2.

The sponsor carried out additional PK/PD studies at the request of the Agency. A sigmoid Emax model was developed with data from study 309 (intermittent bolus study) and the model was checked with data from studies 305 and 307. Additionally, the sponsor combined data from

studies 305, 307 and 309 and checked the model with study 311. The following findings were reported:

1. The objective of the analysis was to establish models for the concentration vs. PCWP and concentration vs. systolic blood pressure (SBP) of hBNP. A population approach using NONMEM was used and the pharmacodynamics for studies 305, 307 and 309 were modeled as a sigmoid Emax model.

2. The model predictions were checked against data from study 704.311, with the PK parameter estimates being from study 311 and the PD parameter estimates were obtained from the pharmacodynamic analysis of studies 305, 307 and 309 combined.

3. For PCWP from studies 305, 309 and 307 combined, the geometric means were as follows (the numbers in parenthesis correspond to the 95% confidence intervals):

- Keo=0.38 h^{-1} (0.32, 0.44 h^{-1})
- Half-life for keo=1.83 hours (1.56, 2.43 h)
- Ce50=6.07 ng/ml (5.07, 7.27 ng/ml)
- γ=0.95 (0.86, 1.04)
- Emax=26.3 mm Hg (25.1, 27.7 mm Hg)

4. For SBP from studies 305, 307 and 309 combined, the geometric means were as follows:

- Baseline SBP=113.2 mm Hg
- Emax= 21.2 mm Hg (18.5, 24.3 mm Hg)
- Keo=0.18 h^{-1} (0.15, 0.22 h^{-1})
- Half-life for keo=3.89 hours (3.18, 4.78 h)
- Ce50=3.17 ng/ml (2.96, 3.39 ng/ml)
- γ=1.19 (0.95, 1.51)

VII. Effect of age:

Age was not correlated with Natrecor CL using both a linear regression and a population PK approach (studies 704.325 and 704.311).

VIII. Effect of renal function:

Study 704.325 showed that there may a trend towards a direct relationship between Natrecor plasma CL and creatinine clearance and an inverse relationship between Natrecor plasma clearance and serum creatinine, using linear regression analysis.

Study 704.311 suggested that creatinine clearance was a significant covariate for plasma clearance of Natrecor, when the model for clearance did not include other covariates, particularly body weight. Although creatinine clearance was not included in the final model for hBNP clearance, due to the confounding nature of (calculated) creatinine clearance and weight, and the fairly limited range of creatinine clearance in this population (15 subjects had values of 30 to 49 mL/min and 5 had values < 30 mL/min), the possibility that renal function influences hBNP clearance cannot be ruled out. For these data, there is an approximately 99% chance that hBNP clearance decreases no more than approximately 10.9% with each 10 mL/min decrease in creatinine clearance.

IX. Effect of gender:

Gender was not correlated with Natrecor CL using both linear regression and population PK analysis (Studies 704.325 and 704.311). The clearance in males and females was 8.84 ml/min/kg and 7.78 ml/min/kg, respectively.

X. Effect of disease state (CHF):

Disease state (New York Heart Association CHF Classification) was not correlated with Natrecor CL using both linear regression and population PK analysis (Studies 704.325 and 704.311).

XI. Effect of ethnicity

The clearance of hBNP was not significantly different in Blacks, Whites and Hispanics (Study 704.325). In Blacks, the clearance was 7.95 ml/min/kg (n=18), in Whites, the clearance was 9.00 ml/min/kg (n=39) and in Hispanics, the clearance was 7.40 ml/min/kg (n=8).

XII. Effect of hBNP production method

No differences were noted in the plasma clearance of hBNP produced by recombinant or synthetic technology as analyzed by linear regression (Study 704.325). The clearance values for the synthetic and recombinant peptides were 8.54 ml/min/kg and 8.50 ml/min/kg, respectively.

XIII. Population PK analysis:

A population PK analysis was carried out (study 704.325 and study 704.311) to determine the effects of demographics and clinical variables on PK parameters. Both studies were in agreement with each other. In study 311, the CL of Natrecor was found to be linearly correlated with body weight ($p<0.001$). Other covariates analyzed, such as ethnicity, method of production of Natrecor, PCWP, CI and SVR did not have a significant influence on the clearance of Natrecor.

In the final model:

- Clearance was found to be linearly correlated with body weight ($p < 0.001$) and was estimated (without the outliers) to be 9.5 mL/min/kg (95% confidence interval: 7.9 mL/min/kg, 11.1 mL/min/kg).
- Interindividual variability of CL was estimated to be 55% (95% confidence interval: 42%, 65%).
- endogenous hBNP plasma concentration was estimated to be 610 pg/mL (95% confidence interval: 443 pg/mL, 777 pg/mL)
- interindividual variability of the endogenous hBNP concentration was estimated to be 90% (95% confidence interval: 71%, 107%)
- The intraindividual and residual variability was estimated to be 26% (95% confidence interval: 20%, 31%).

XIV. Assay:

The assay used to study the plasma levels of hBNP in clinical trials was an ELISA.

The lower limit of quantitation for this assay was pg/ml. This assay did not distinguish between Natrecor and the endogenous hBNP, since their amino acid sequences were identical.

XV. Formulation:

Chemical Component	Supplier	Quantity Per Typical Batch[b]	Quantity Per mL of Drug Bulk Solution	Quantity Per Vial After Lyophilization	Concentration After Reconstitution[a]
hBNP Drug Substance			mg/mL		
Mannitol, USP			20.0 mg/mL		
Sodium Citrate, Dihydrate, USP			2.94 mg/mL		
Citric Acid, Monohydrate, USP			2.10 mg/mL		
					NA
					NA

XVI. Metabolism and excretion based on preclinical studies

It is believed that the natriuretic peptide clearance (NP-C) receptor is involved in the metabolism of atrial natriuretic peptide (ANP). NP-C is a cell surface receptor that binds both ANP and BNP and mediates the internalization of the peptide and delivery to lysosomes where the peptide is hydrolyzed to inactive fragments and individual amino acids. Therefore, at least in rabbits, the NP-C receptor is involved in the elimination of BNP.

Additionally, peptidases such as neutral endopeptidase 24.11 (NEP 24.11), present in the vascular lumen play a role in the metabolism of peptide hormones like ANP and BNP. The inhibition of NP-C or NEP has led to an approximately two-fold increase in circulating hBNP in rabbits.

Comments to the Medical Division:

Comments communicated to the to the Sponsor prior to the advisory committee meeting on January 29th, 1999:

1. No QC samples were provided for any of the studies. The sponsor is advised to submit QC samples for each individual study.

2. PK analysis conducted in study 704.325 using linear regression analysis do not show a correlation between Natrecor plasma clearance and body weight, however an analysis conducted using NONMEM in study 704.311 shows a linear correlation between plasma clearance of Natrecor and body weight. This discrepancy should be addressed by the sponsor.

3. The sponsor asserts that there is no apparent delay in response after plasma hBNP concentrations has reached a steady-state. While the observations do not allow for visual inspection of this feature an alternative model can be tested against the currently proposed PK/PD model. A hypothetical biophase steady-state concentration is used to relate concentration to effect as: $C_e^{ss} = C_p^{ss} \cdot (1 - \exp^{-k_{e0} \cdot t})$, where, k_{e0} represents the dissociation constant of the drug-receptor binding. Determining the k_{e0} allows the appreciation of the onset/offset properties of the PD effect. The sponsors analysis of hemodynamic responses at early (after 1 and 1.5 hours) and delayed (after 2.5 and 3.0 hours) times following escalation to the 0.03 µg/min/kg dose level in 6 subjects showing no significant difference in responses measured between early and late times, suggests that the pharmacodynamics have achieved equilibrium after one hour of constant infusion. It is clear that exogenous doses of hBNP show beneficial therapeutic effects in the management of CHF. The concentration-response relationship was shown to be graded, suggesting that the dose can be individualized within the range 0.003 to 0.1 µg/min/kg. Endogenous

levels of hBNP are raised in CHF and mediate compensatory mechanisms in the regulation of cardiovascular homeostasis and therefore, it may be worthwhile to model the baseline hBNP with a sigmoid Emax model as well as the exogenous hBNP. It may be assumed that the potencies of endogenous and exogenous hBNP are the same or different.

Comments made to the sponsor during a meeting held after the advisory committee meeting on February 5th, 1999:

1. The modeling conducted with study 704.307 does not correlate with the PD seen in study 704.311. In other words, in study 704.311 (infusion study over 24 hours), a delay was seen in the onset of the effect (PCWP) and similarly, there was a delay in the offset of the pharmacodynamic effect. These phenomena were not accounted for in the modeling conducted with data from study 704.307 (an incremental infusion study where the doses were increased every 1.5 hours). The measurements in study 704.307 were carried out at pharmacokinetic steady state and thus did not allow the establishment of a lag period in either the onset or offset of pharmacodynamic effect. Therefore, study 704.307 was not optimal for the development of a PK/PD model. However, study 704.311 was much better suited for the development of a PK/PD model, and allowed for the determination of a lag between plasma levels and drug action, since pharmacokinetic and pharmacodynamic measurements were undertaken during both the initial phase of drug distribution to steady state as well as during the offset of drug effect after the stop of infusion (measurements in both PK and PD were made up to 4 hours after the stop of Natrecor infusion).

2. Study 704.311 was deemed by the FDA to be well suited for the development of a PK/PD model because it allowed the sponsor to answer the questions regarding the lag time involved in the onset of drug effect. In fact, during the meeting on February 5th, 1999, the sponsor informed the division that a new model was currently being developed using data from study 704.309 which is an incremental bolus dose study. The sponsor informed the Agency that with the new model developed, a lag time was observed between the plasma levels and the pharmacodynamic effect, specifically the PCWP. The lag time was estimated at 2.5-3 hours. The model was validated with data

from study 704.311. The Agency is currently awaiting the study report for the above-mentioned PK/PD analysis which will be reviewed as an amendment to this review.

3. The sponsor did not investigate whether there was tolerance in the PD response to the effects of Natrecor. The medical officer made an observation that the effect of the drug seemed to decrease between the 6 and 24 hour time points. In order to establish whether this decrease in the effect is due to a decrease in drug clearance or whether there is in fact a tolerance phenomenon, the sponsor was asked to use the predicted model to address this issue.

4. There is serious concern regarding the adverse effects of Natrecor which occur as an extension of the pharmacologic effect of the drug. Of particular concern is the observed hypotension. The sponsor was asked to incorporate hypotension into the PK/PD model that is currently being developed in order to determine if there is a concentration-effect relationship for this safety parameter. Therefore the sponsor should model the hypotension effect of Natrecor as one of the PD endpoints and determine at what plasma concentrations does the hypotension occur and what is the time course for the onset and offset of this adverse event.

5. Due to the adverse event profile of Natrecor, the division is currently considering lowering the starting dose of the drug to 0.0075 µg/kg. However, this dose has not been tested for efficacy. Therefore, if a PK/PD is successfully developed, the sponsor should be able to predict the efficacy of the drug at the lower dose. Additionally, the sponsor is considering changing the dosage regimen to omit the bolus dose of Natrecor. This too has not been tested in the pivotal clinical studies, therefore, the sponsor should also try to address this issue with the PK/PD model being developed.

6. The medical reviewer has recently raised a new concern regarding possible differences between the recombinant protein formulation and the synthetic peptide formulation of Natrecor. In study 704.325, using linear regression analysis, no correlation was found between clearance and the source of Natrecor. However, the medical team leader, Dr. Karkowsky, believes that preclinical data indicate that there may in fact be differences between the Natrecors prepared by 2 different routes, ie, recombinant protein and synthetic peptide methodologies. The sponsor can address this issue of possible

differences in the activities of hBNP produced via the 2 different routes by pooling data from studies 704.311 and 704.325 (the only study submitted to the OCPB reviewer which contains data with the recombinant hBNP). After pooling, these data could be analyzed using a population PK approach.

Comments regarding the additional PK/PD analysis conducted by the sponsor and submitted on March 12th, 1999, in response to above-mentioned concerns:

1. The PD parameters obtained from the modeling study indicate that a 50% decrease in systolic blood pressure will be reached at half the plasma concentrations of hBNP (Ce50=3 ng/ml) as compared to a 50% decrease in PCWP (Ce50=6 ng/ml).

2. Additionally, based on the values for keo, it appears that the half-life for the lag in decrease of systolic blood pressure is twice as long as that for a decrease in PCWP. This indicates that once hypotension is observed, it will take twice as long to recover from the adverse event as it would to return to baseline PCWP values. Therefore, based on the severity of the hypotension, one may have to wait 20 hours (5 times 4 hours) in order to return to steady state for hypotension, before one can attempt to dose with either Natrecor or another drug for CHF.

3. When the predictions from the model developed with combined data of studies 305, 307 and 309 was checked with the observed PD data from study 311, neither predicted PCWP nor predicted SBP fell within the 95% confidence intervals of the mean observations for the lowest dose tested (0.015 µg/kg/min). The sponsor attributes this to an "abnormal" dose-response relationship in study 311, where the response (decrement in PCWP and SBP) were greater at 0.015 µg/kg/min versus the values at 0.03 µg/kg/min.

4. The model "reasonably" predicts the decrease in PCWP and SBP for the first 6 hours of infusion of hBNP at the 0.03 and 0.06 µg/kg/min infusion rate. However, the model did not predict the mean observations for neither the PCWP nor the SBP during the offset phase of the dynamic effect, ie, at and after the 24-hour time point. Tolerance to the effects of hBNP cannot be ruled out at this point.

But since tolerance was not built into the model, conclusive evidence regarding tolerance does not exist. However, there does appear to be an attenuation of the response for PCWP at time points beyond 10 hours.

5. Simulation studies indicate that bolus doses less than 1 μg/kg would contribute little to a decrement in PCWP despite an immediate increase in plasma hBNP levels. With a bolus dose of 3 μg/kg, the values for PCWP are at 90% of steady state (24 mm Hg) at 50 minutes, and at 50% of steady state at 10 minutes post dose.

6. Simulation studies also show that whilst the decrease in SBP seems to lag behind the decrease in PCWP (due to a larger keo for SBP decrease), the recovery back to baseline after the stop of infusion will take twice as long for SBP, as compared to PCWP. This means that if a patient develops hypotension during dosing with Natrecor and the infusion is stopped because of this adverse event, the PCWP values will return to baseline pre-Natrecor levels twice as fast as the return of the blood pressure to baseline levels. In other words, a patient may be suffering from hypotension concurrently with a high PCWP resulting from CHF.

/S/

Nakissa Sadrieh, Ph.D.

Date: 3/25/99

/S/

RD/FT Patrick Marroum, Ph.D. Date: 3/25/1999

Biopharm day: March 16th, 1999 (Sadrieh, Marroum, Robbie, Dorantes, Mehta, Lesko, Huang, Karkowsky, Throckmorton, Miller, Lazor, Selen, Chen, Kumi, Gobburu)

CC List: NDA 20-920, HFD-110 (Willard); HFD-860 (Sadrieh, Marroum), HFD-340 (Wiswanathan).

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Pharmacokinetic analysis of plasma concentrations of Natrecor hBNP from study 704.305 [IND] "A phase I/II double-blind, randomized, placebo-controlled ascending dose study of the hemodynamic and renal effects of a single intravenous bolus of Natrecor hBNP in subjects with congestive heart failure".

Study No. 704.305 **Volume 1.31** **Pages 80-143**
Report No. DAD 94-23

Investigators: Robert E. Hobbs, MD (Cleveland Clinic Foundation)
Leslie Miller, MD (St. Louis University Medical Center)

PK data analyzed by: Nancy Sambol, PharmD (UCSF)

Medication:

Natrecor: 5 mg lyophilized powder reconstituted in 5% dextrose in water (lot # E00013A1). The Natrecor was produced by synthetic peptide methodology.

Placebo: 5% dextrose in water.

Dose level:

Single IV bolus dose of:

0.3 µg/kg (n=4)
1 µg/kg (n=4)
3 µg/kg (n=4)
10 µg/kg (n=3)
15 µg/kg (n=4)
20 µg/kg (n=4)

In each dose group, an additional person was assigned placebo.

Study population:

Thirty subjects aged 18 years or older, who were diagnosed with New York Heart Association (NYHA) Class II, III or IV CHF.

Design:

Randomized, double-blind, placebo-controlled Phase I/II trial in 30 subjects. Administration of study drug began with the 0.3 μg/kg dose. Dose administration was increased incrementally, with placebo doses interspersed.

Blood was collected at the following time points: 1 minute prior to dosing, 2, 5, 10, 15, 30, 60 and 90 minutes post dosing.

Assay procedures:

Please refer to Appendix 9 for details about the assay. The methodology used was an ELISA and was described previously. Briefly,

The LOQ was pg/ml.

Data analysis:

To adjust for endogenous hBNP concentration in the subjects given Natrecor, the pre-dose concentration of Natrecor was subtracted from each post-dose concentration. The within-subject endogenous hBNP concentrations were relatively constant during the 90 minutes of the study (as determined from the subjects receiving placebo).

Both a one- and two-compartment open model with instantaneous input were fit to the data using

The dependence of Vss and CL on dose was investigated by combining the data from 0.3 and 1 μg/ml dose groups (low dose), the 3 and 10 μg/ml groups (medium dose) and the 15 and 20 μg/ml groups (high dose) and comparing the 3 groups by ANOVA. This was done to increase sample size and thus statistical power of the analysis for detecting difference.

Results:

Of the 30 subjects, 23 were given Natrecor. Of these 23, data from 12 of the subjects (52%) were described by a two-compartment model, whereas data from 11 of the subjects were described by a one-compartment model.

In those subjects to whom a two-compartment model was applied, $t_{1/2\alpha}$ was equal to 1.4 minutes (SE=0.3) and the first phase of the concentration decline accounted for 30% of the total AUC. The estimate of $t_{1/2\beta}$ or t ½ was 20.2 minutes (SE=1.8 minutes). The estimate of Vc was 0.072L/kg (SE=0.010 L/kg) and the estimate of Vss was 0.130 L/kg (SE=0.018 L/kg). Clearance was estimated at 5.4 ml/min/kg (SE=0.4 ml/min/kg). There was no dose-dependence in Vss or CL. It is suggested that additional samples at early time points (immediately post-dose) be taken for future pharmacokinetic studies.

The following table lists all the PK parameter estimates of hBNP after a single IV bolus dose:

Table 1

Pharmacokinetic Parameter Estimates (mean ± SE) of hBNP in Subjects with Congestive Heart Failure after a Single Intravenous Bolus Dose

Parameter	All Data	0.3 μg/kg**	1 μg/kg	3 μg/kg	10 μg/kg	15 μg/kg	20 μg/kg
A (pg/mL)	NA***	5,260 ± 594	114,184 ± 59846	341,547 ± 168,299	—	1,782,626	840,215 ± 63,079
α (min^{-1})	0.845 ± 0.206	0.405 ± 0.084	0.982 ± 0.370	0.742 ± 0.124	—	1.973	1.03 ± 0.71
Area-A/α (%)	30 ± 4	25 ± 6	43 ± 23	38 ± 8	—	38	19 ± 3
B or C_0* (pg/mL)	NA	1,516 ± 709	7,421 ± 2,581	26,944 ± 3,249	98,296 ± 11,709	102,663 ± 25,831	115,547 ± 21,480
β or k* (min^{-1})	0.041 ± 0.004	0.024 ± 0.004	0.041 ± 0.011	0.045 ± 0.006	0.045 ± 0.006	0.055 ± 0.013	0.034 ± 0.002
Area-B/β (%)	70 ± 5	75 ± 6	57 ± 23	62 ± 8	—	62	81 ± 3
$t_{1/2,\alpha}$ (min)	1.4 ± 0.3	2.1 ± 0.7	0.8 ± 0.3	1 ± 0.2	—	0.4	1.6 ± 0.6
$t_{1/2,\beta}$ or $t_{1/2}$* (min)	20.2 ± 1.8	31.3 ± 5.6	20.8 ± 5.2	15.8 ± 1.5	16.0 ± 1.9	15.9 ± 4.6	20.7 ± 1.3
V_c or V* (L/kg)	0.072 ± 0.010	0.054 ± 0.007	0.052 ± 0.022	0.062 ± 0.032	0.105 ± 0.014	0.098 ± 0.035	0.071 ± 0.025
V_{ss} or V* (L/kg)	0.130 ± 0.018	0.216 ± 0.088	0.098 ± 0.025	0.080 ± 0.023	0.105 ± 0.014	0.136 ± 0.019	0.141 ± 0.024
CL (mL/min/kg)	5.40 ± 0.40	6.08 ± 1.58	4.48 ± 0.26	4.32 ± 1.05	4.57 ± 0.19	7.30 ± 0.44	5.45 ± 0.81
k_{12} (min^{-1})	0.485 ± 0.152	0.216 ± 0.054	0.422 ± 0.017	0.369 ± 0.034	—	1.19	0.727 ± 0.581
k_{21} (min^{-1})	0.101 ± 0.015	0.101 ± 0.039	0.068 ± 0.020	0.094 ± 0.014	—	0.065	0.138 ± 0.023
k_{10} (min^{-1})	0.288 ± 0.080	0.112 ± 0.027	0.518 ± 0.379	0.317 ± 0.105	—	0.747	0.198 ± 0.107

* One-compartment model.

** Includes 1 subject given approximately 0.5 μg/kg.

*** NA = not applicable (value depends on dose).

Conclusions:

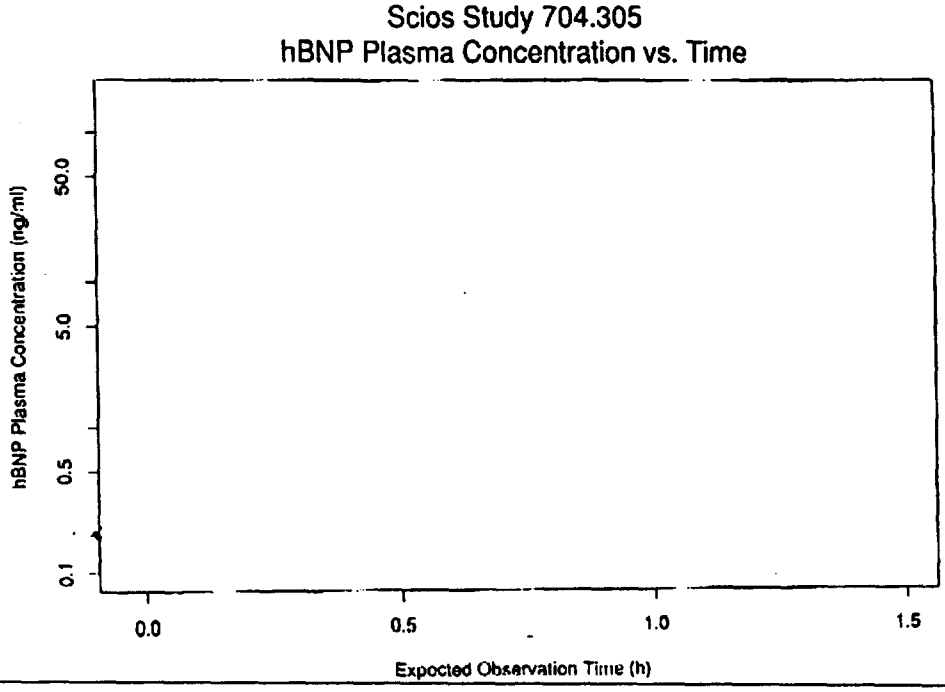
The present study was an IV bolus study conducted in patients with congestive heart failure. The doses used were 0.3, 1, 3, 10, 15 and 20 µg/kg Natrecor.

Data from half the subjects were described by a 2 compartment model, whereas the data from the other half of the subjects were described by a one compartment model.

In those subjects to whom a two-compartment model was applied:

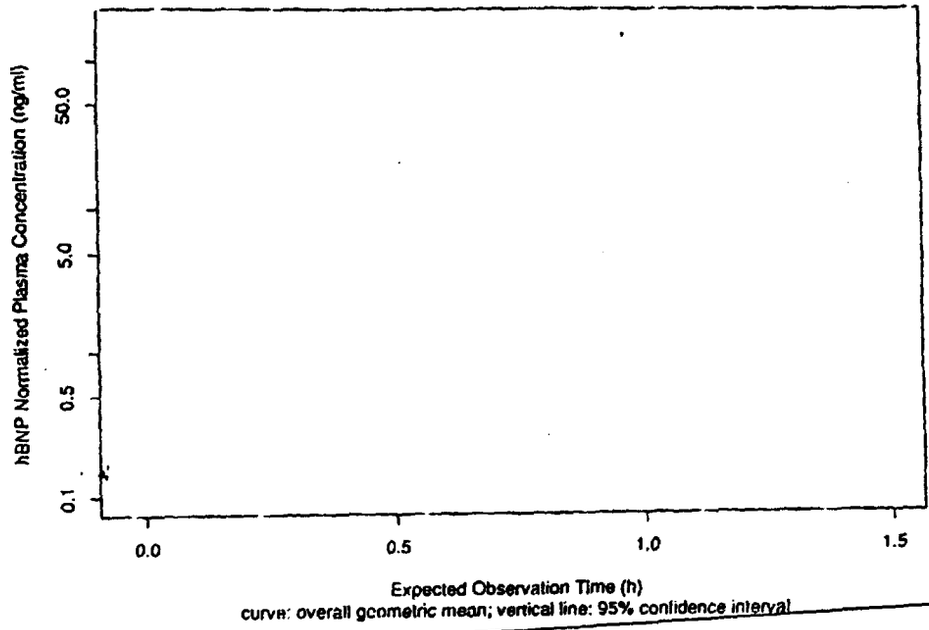
$t_{1/2\alpha}$ =1.4 minutes (SE=0.3)
The alpha phase concentration accounted for 30% of the total AUC
The estimate of $t_{1/2\beta}$ =20.2 minutes (SE=1.8 minutes)
The estimate of Vc was 0.072L/kg (SE=0.010 L/kg)
The estimate of Vss was 0.130 L/kg (SE=0.018 L/kg)
Clearance was estimated at 5.4 ml/min/kg (SE=0.4 ml/min/kg)
There was no dose-dependence in Vss or CL

The following figure shows the plasma concentration-time profile for hBNP at the doses used in study 704.305.



The following figure shows the dose-normalized (to the 10 μg/kg dose) plasma concentrations of hBNP from a single IV bolus dose.

Scios Study 704.305
hBNP Plasma Concentration (normalized to 10 mcg/kg) vs. Time



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Pharmacokinetic analysis of plasma concentrations of Natrecor hBNP from study 704.309 (IND)"A phase II randomized, double-blind, placebo-controlled, dose-repsonse study of a 24-hour course of Natrecor hBNP administered as an intermittent intravenous bolus in subjects with congestive heart failure".

Study No. 704.309 **Volume 1.31** **Pages 144-247**
Report No. DAD 96-03B

Report date: November 12th, 1997 (revised)

PK data analyzed by: Nancy Sambol, PharmD (UCSF)
Chui Yu Lui

Objectives:

To assess the effects of a 24-hour course of Natrecor hBNP, administered as an intermittent bolus, on central hemodynamic parameters and to determine the pharmacokinetic profile of the drug.

Medication:

Natrecor: 5 mg lyophilized powder reconstituted in 5% dextrose in water (lot # E00013A1 and G0003A1). The Natrecor was produced by synthetic peptide methodology.

Placebo: 5% dextrose in water.

Dose level:

Single IV bolus dose of:

5 µg/kg every 4 hours for 24 hours (n=15)
10 µg/kg every 6 hours for 24 hours (n=14)
10 µg/kg every 4 hours (n=15)

Sixteen subjects were assigned to placebo.

Study population:

Sixty subjects aged 18 years or older, who were diagnosed with New York Heart Association (NYHA) Class II, III or IV CHF.

Design:

Randomized, double-blind, placebo-controlled Phase II dose-response study.

Blood was collected at the following time points: 15 minutes prior to dosing, 2, 5, 15 minutes, 1 and 1.5 hours after the first and last dose.

Assay procedures:

Please refer to Appendix 9 for details about the assay. The methodology used was an ELISA and was described previously. Briefly,

The LOQ was pg/ml. Quality control samples were not provided for this or any other study reviewed herein.

The mean concentration of endogenous hBNP is reported to be highly variable amongst subjects and it is also reported to be variable within a subject. To adjust for the presence of endogenous hBNP, the pretreatment concentration of hBNP was subtracted from hBNP concentrations measured in post-dosing samples.

Data analysis:

The PK parameters were measured using model-independent methods. The reason for this choice of data analysis is reported to be the lack of enough concentration-time points for a two-compartment model.

Results:

The following mean estimates were calculated and include the 95% confidence intervals in parentheses:

CL:	9.8 ml/min/kg (7.8, 11.8 ml/min/kg)
Varea:	0.24 L/kg (0.20, 0.29 L/kg)
Vss:	0.21 L/kg (0.18, 0.24 L/kg)
MRT:	0.38 hours (0.34, 0.41 hours)
Parameter k:	First dose: 2.53 h^{-1} (2.27, 2.78 h^{-1})
	Last dose: 2.57 h^{-1} (2.26, 2.80 h^{-1})
	Overall estimate: 2.55 h^{-1} (2.38, 2.71h^{-1})
Half-life:	First dose: 0.30 hours (0.27, 0.32 hours)
	Last dose: 0.29 hours (0.27, 0.32 hours)
	Overall estimate: 0.29 hours (0.28, 0.31 hours)
Cmax:	First dose: 36.5 ng/ml (29.6, 43.3 ng/ml)
	Last dose: 38.4 ng/ml (29.2, 43.7 ng/ml)
	Overall estimate: 37.4 ng/ml (32.7, 42 ng/ml)

The values for Cmax have been normalized to the 5 µg/kg dose.

It should be noted that the variability in these parameters is rather large. The %CV reported ranges from 26.6 (t ½ first dose) to 65.8 (CL).

There is no significant difference in k, t ½ or Cmax (normalized to the 5 µg/kg dose) between the first and the last doses. The mean difference between the last and the first dose values for k is 0.03 h^{-1} , for t ½ it is – 0.01 hours and for the normalized Cmax it is 3.8 ng/ml.

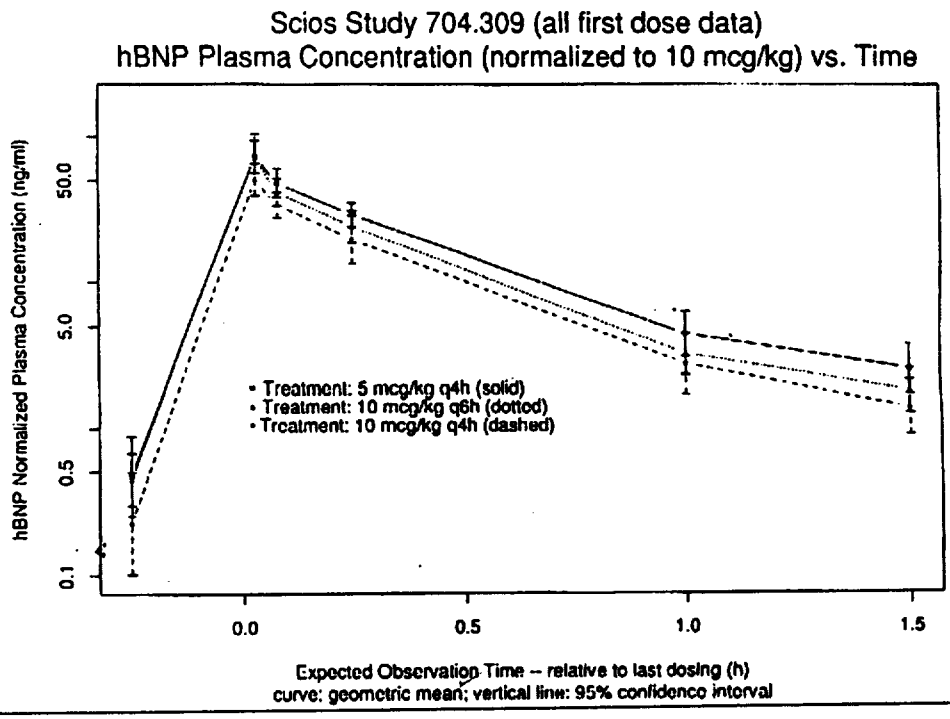
Conclusions:

This study was an intermittent IV bolus study in which subjects with congestive heart failure were administered 5 µg/kg of Natrecor every 4 hours or 10 µg/kg Natrecor every 4 or 6 hours. The mean parameter estimates, which were calculated by a model-independent (non-parametric) method, are the following:

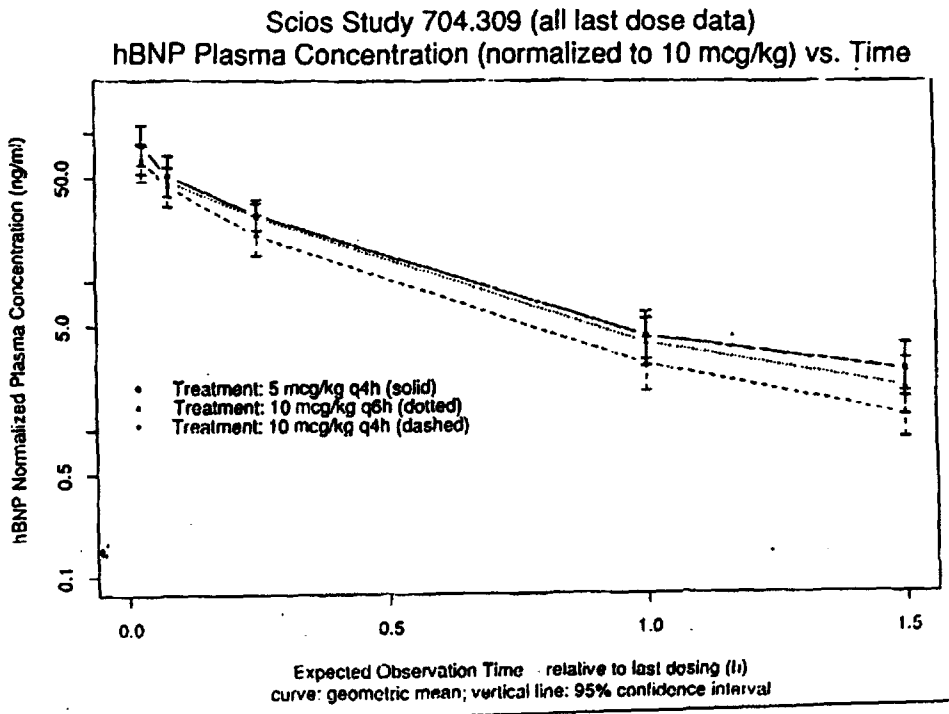
CL:	9.8 ml/min/kg (7.8, 11.8 ml/min/kg)
Varea:	0.24 L/kg (0.20, 0.29 L/kg)
Vss:	0.21 L/kg (0.18, 0.24 L/kg)
MRT:	0.38 hours (0.34, 0.41 hours)
Parameter k:	Overall estimate: 2.55 h^{-1} (2.38, 2.71h^{-1})
Half-life:	Overall estimate: 0.29 hours (0.28, 0.31 hours)
Cmax:	Overall estimate: 37.4 ng/ml (32.7, 42 ng/ml)

No significant difference was noted in the estimates of the first and last dose parameters. The sponsor reports that since the t ½ is 20 minutes and the dosing interval in the study was 4 or 6 hours (12-18 times the t ½), the plasma concentration at the end of the dosing interval is expected to be negligible. Therefore, since the difference between the first and last dose Cmax values is insignificant, this suggests that the central volume of distribution does not differ between the first and last bolus doses. Consequently, the lack of difference in the values for the first and last dose values for k and t ½ also suggests that clearance does not change over time with intermittent bolus dosing over 24 hours.

The following figure shows the hBNP plasma concentration-time profile after the first dose of hBNP from an intermittent bolus dose study.



The following figure shows the hBNP plasma concentration vs time profile after the last dose of the intermittent bolus dosing regimen:



Appendix 3

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Pharmacokinetic analysis of plasma concentrations of Natrecor hBNP from study 704.310 (IND [redacted])"A phase II randomized, double-blind, placebo-controlled, ascending dose-repsonse study of a 24-hour course of Natrecor hBNP administered as an intermittent intravenous bolus in subjects with congestive heart failure on angiotensive converting enzyme inhibitor therapy".

Study No. 704.310 **Volume 1.31** **Pages 248-321**
Report No. DAD 96-03C

Report date: November 7th, 1997 (revised)

PK data analyzed by: Nancy Sambol, PharmD (UCSF)
Chui Yu Lui

Objectives:

To assess the effects of a 24-hour course of Natrecor hBNP on central hemodynamic parameters and to determine the pharmacokinetic profile of the drug in subjects with CHF who were receiving ACE inhibitors.

Medication:

Natrecor: 5 mg lyophilized powder reconstituted in 5% dextrose in water (lot # E00013A1 and G0003A1). The Natrecor was produced by synthetic peptide methodology.

Placebo: 5% dextrose in water.

Dose level:

Single IV bolus dose of:

3 µg/kg every 4 hours for 24 hours
5 µg/kg every 4 hours for 24 hours
10 µg/kg every 4 hours

Forty-three subjects received Natrecor, 31 of which were also receiving concomitant treatment with ACE inhibitors. Seventeen subjects were